

*Dear recommender and reviewers,*

*We are pleased to submit our revised manuscript “Multiple hosts, multiple impacts: the role of vertebrate host diversity in shaping mosquito life history and pathogen transmission”. We are grateful to the recommender and the reviewers for their meticulous and relevant comments. You will find below all the changes undertaken.*

*We thank you again for considering and reviewing our manuscript.*

*Yours sincerely,*

*Amélie Vantaux on behalf of all coauthors*

### **Recommender comments**

*All changes made in response to the recommender’s comments are highlighted in blue in the revised version of the ms.*

1/ Lines 107-108. I suggest including a map showing the exact location of where the samples come from? Not everyone is well versed in geography and West Africa is a large region with many countries.

*Thanks for the suggestion. The geographic situation of the sites is now presented in Figure 1 in the new version of the ms.*

2/ Line 119. Out of curiosity. Why did you use thick blood smears instead of thin ones? I ask because for wild animals malaria we use thin blood smears. Can you explain the pros of thick blood smear vs. thin blood smears?

*The thick blood smears allow to analyze a larger volume of blood and thus to also detect low parasitemia individuals. Thin blood smears are more adapted to determine and count the different parasite stages, however they will miss a proportion of low density individuals.*

3/ Lines 127-128. I know you are citing a reference indicating that heat inactivation works. But what steps if any did you take to ensure that gametocytes were inactivated? That is, did you take a small blood sample and conducted some type of test on gametocytes to determine if they were active or not?

*We did not take a small volume of blood and conduct some type of test on gametocytes to determine if they were active or not. The heat-inactivation technique has been validated in the cited reference and has been used in many experiments carried out in this laboratory (PMID: 27490374; PMID: 35687594; PMID: 27827429; PMID: 27146309; PMID: 24850924). The inactivation is regularly verified by dissecting a subset of uninfected mosquitoes on day 7 post-blood meal to confirm that no parasites are observed in the*

*mosquito midgut. However, we did not do it in this experiment as an important proportion of unfed females was already removed at each blood-meal and we decided to maximize sample sizes used for the experiments themselves.*

4/ Line 132. But if you used 6 different parasite isolates, can you really say they are replicates? What do you mean by "six distinct parasite isolates"? Are they the same parasite strain or how are they distinct from each other?

*They are biological replicates and not technical replicates. We are working with field samples therefore we are considering the six blood samples withdrawn from the six human volunteers to be 6 distinct parasite isolates which is likely the case in an area of high malaria endemicity such as Burkina Faso where multiplicity of infection is high (PMID: 33903595; PMID: 29738740; PMID: 32819420). That would likely be different in a low transmission setting where parasite diversity tend to decline and strains tend to be more homogenous. However, we have not genotyped our samples to determine the parasite genetic diversity and we are now specifying this in the methods (lines 137-139).*

5/ Line 135. Figure 1. What is the difference between replicates in cups and those in cages? Would not the use of different containers affect the replicability of your experiments? Also, why for replicates 3-5 the only trait measured was competence? Finally, in the top figure (A), why there are three cylinders numbered 1,2,3? What does cylinders represent? It is confusing given that in section (B) of the figure there are only two cages. It is important to explain details of the experimental design in the figure's legend in order to avoid confusion for the reader.

*Replicates in cups allowed to follow several life history traits (competence, survival, fecundity, F1 development time and size) and by having 121 cups we ensured enough technical replicates to give sensible results. However, the number of individuals available to measure competence at the oocyst and the sporozoite stages (119 and 34 individuals respectively) was rather reduced owing to both mortality and loss of individuals at each blood meal (unfed females were discarded). The replicates in cages allowed to follow much more females, however, measuring traits such as fecundity would give a very gross estimate using such a large number of individuals to calculate the average number of eggs per female for example. Thus, this design was used to measure competence only, on a larger number of individuals, and allowed us to screen 757 individuals at the oocyst stage and 235 individuals at the sporozoite stages, giving more robustness to our results. Finally, the two designs do not affect replicability as they were analyzed independently. We have now made this clearer (lines 141-147, 156-162 and 257-258).*

*We acknowledge that having 3 cylinders representing cups was confusing, we have corrected this and have explained into more details the experimental design in the figure's legend and in the main text (lines 141-147, 156-162).*

6/ Line 143. What does it mean venous blood?

*Venous blood means blood withdrawn from a vein as opposed to capillary or arterial blood. Although they are less common we prefer to specify as some experiments sometimes use capillary blood for example.*

7/ Line 167. The word "group" in the phrase "...the first three group..." must be plural "groups"

*This has been corrected (line 187).*

8/ Line 177. Please define each acronym you use at first appearance. I guess that "dpbm" is "days post blood meal", but not every reader will be able to guess it. Please spell out on first appearance.

*We have now spelled it in the brackets (line 190-191).*

9/ Line 185. I am not 100% sure, but I think the right word is "thoraxes", please check the correct spelling of the plural

*We checked and both spellings are correct.*

10/ Lines 185-186. Did you extract salivary glands? If so, did you use one gland to do microscopy and check for the presence of sporozoites and the other gland for PCR?

*We did not extract salivary glands. We crushed the head and the thorax of each individual before carrying out the PCR, which is a classical metric in malaria transmission studies (PMID: [26674956](#)). We have reformulated our sentence to be more precise (lines 206-208).*

11/ Line 249. Why in this model parasite isolate was coded as fixed factor and not as a random factor? It is not clear, please explain.

My guess is that it has to do with the fact that the experimental design in figure 1 is not completely clear as I explained above. That is, the first set of experiments showing three cylinders representing I suppose 3 replicates, and the second set of experiments only one cage per treatment is shown.

*There are only two parasite isolates in the first experimental design which preclude using isolate as a random factor. We have now better explained figure 1 and the experimental designs we used as requested in comment #5 (lines 141-147, 156-162 and 257-258).*

12/ Line 253. Can you please provide a rationale for giving 0 to the unfed females? What do you mean by "censoring" status? If they were unfed, they should not be included in the analysis, right?

*After each blood meal, unfed females were removed as the measurements of the fecundity parameters would be biased otherwise. Those females can be considered as censored i.e. as lost to follow-up. Ignoring the censored observations results in the loss of potentially valuable information on survival and an underestimation of the probability of survival beyond the fixed time point (PMID: [8908598](#)). The underestimation will depend on the proportion of censored observations in the data. Therefore, to not lose valuable information and following the definition from the R Book from Crawley we used 'survival models with a censoring indicator (1 indicates that the response is a time at death, 0 indicates that the individual was alive when last seen'). We have now better explain why unfed female were given a censoring status of 0 (lines 275-276).*

13/ Line 258. Why did you choose the quasipoisson structure for these analyses? Is it because you could not fit a regular Poisson?

*Our data are overdispersed therefore we compensated for the overdispersion by refitting the model using quasi-Poisson errors. We have now specified this lines 280-281.*

14/ Line 268. Please remove one of the "and" of the sentence, there are two  
*We removed the extra "and" line 314.*

15/ Lines 270-272. Why did you choose to do model selection doing stepwise removal of terms? As far as I know, stepwise removal can lead to errors because the order in which terms are removed or entered (in stepwise addition) matters.

I may be wrong, but I think a better approach to model selection would be to use an information criterion like Akaike, where you can build your different models - from a null model with no variables up to a complete model - and then select those with the best AIC. Perhaps the stepwise removal of terms can be left for models like the quasiPoisson ones, where you cannot actually obtain an AIC because of the forcing of the model structure. Although this is just a suggestion, I encourage you to do this as a way of comparison to see if your results do not change when using the information theoretic approach.

*AIC and likelihood ratio test (LRT) have different purposes, AIC is useful when the goal is to do model selection for forecasting whereas LRT can be used for significance testing. AIC has the advantage of comparing different models without the models being nested, and it allows informal comparisons of models with different number of parameters. However, while it is freed from the p-value and all the considerations attached to using a p-value of 0.05 it also does not tell much on the significance of a factor. Rules of thumb exist to differentiate models with different AIC, 0-2: similar model, 2-7: different, 7- and more: totally different models, it only gives qualitative differences. We would not be able to give quantitative values on the factors tested, which is the case with LRT. We agree that stepwise removal can lead to errors as the order matters, however how the order terms are removed is also directed by some rules and still aims at having the most parsimonious and adequate model.*

*Concretely to address your concern, we used the package MuMIn and the function dredge which ran a global model and all the possible subset models and from which can be retrieved the AIC values. This has the advantage of avoiding the possible errors of term removal as it is not linked to stepwise removal or addition of terms. Although this should be avoided as the model investigation should be done based on good empirical or theoretical support to avoid 'mistreatment of interactions, neglecting assumptions and several other issues' (<https://doi.org/10.1007/s00265-010-1040-y>). All models for which this function could be ran are presented in the table at the end of this document and it can be seen that for almost all of them the model with the lowest AIC was also the minimal model selected by the LRT approach and that for the 3 cases for which this was not the case their AIC value was in the 2 deltaAIC range for which models are usually considered similar (which is understandable as deltaAIC and P values are based on the same statistical information <https://doi.org/10.1890/13-0590.1>).*

16/ Line 287. Please write "...mosquitoes are..." instead of "...mosquitoes is..."  
*We corrected by "the mosquito is..." (line 309).*

17/ Lines 290-292. This explanation of number of successful feeding attempts is not clear. You say that the number of days spent in state F following the duration of Plasmodium incubation period was counted as the number of successful feeding attempts. However, you have three states representing F, HS, BF, and R, from which only BF can represent a feeding attempt. So, how the number of days spent in state HS or R would add to F? Also, I strongly recommend to prepare a figure showing the model structure, so it is easier to follow your explanations of the simulations. Ideally, the flow diagram should include variables represented as circles and the parameters of the model adding or subtracting to each variable whousl be representing as incoming or outcoming arrows, respectively. I suggest bringing Fig S5 into the main text.

*We agree that the explanation was not clear as there was no state F but only BF which represents a successful feeding attempt. This has now been corrected (line 313). We have now brought the Figure, the Tables and the explanations of the theoretical model in the main text following the comment below. We have also modified the figure explaining the theoretical modeling to include a flow diagram as well as examples of possible sequences of physiological states an individual mosquito could be observed in. Modifications lines 319-383.*

Questions on Appendix 4

18/ 1) Line 36. What do you mean by "blood meal were reduced centered"? You are trying to say that you standardized your data to 0 mean and 1 sd?

*Yes this is what we meant. We have added another phrasing (centered and scaled) in the appendix (line 38).*

19/ 2) Line 57. Why the entity of your model is *An. gambiae*? Your work is related to *An. coluzzi*, even when it was formerly recognized as *gambiae*. Please check all the paper and supplementary info to standardize with the correct species name.

*An. coluzzii (previously An. gambiae M form) and An. gambiae s.s. (previously An. gambiae S form) are sister species belonging to the An. gambiae species complex which are found in sympatry and are formally differentiated using molecular tools. As they are found in the same area, the model was developed for An. gambiae s.l. which encompasses An. gambiae s.s. and An. coluzzii. We have checked the paper and ensured An. coluzzii is written everywhere necessary, notably it was corrected line 57 of the Appendix 4 which is now line 346 of the main text.*

20/ 3) Line 94. Did you mean Table S3 instead of Table 1?

*Yes we meant Table S3, however the theoretical modeling explanatory text, figure and table have been brought to the main text following comment # 17 and this is now Table 1 in the manuscript.*

21/ Finally, I would strongly recommend that in addition of including the model figure flow chart, you also bring into the main text all the details about the theoretical model from lines 52 to line 104, including Table S3. These information will help the reader to better understand your simulations. And please also consider my previous comments on this modeling part.

*We have now brought the Figure, the Tables and the explanations of the theoretical model in the main text following comment # 17 (lines 319-383).*

22/ Lines 326-327. Why is it necessary to indicate that gametocytemia was positively correlated to parasite intensity? Is not that what you would expect given that parasite intensity is measured as the number of peripheral blood stages counted in blood smears? or what else did you include in your measure of parasite intensity?

*Parasite intensity is the number of parasites in the mosquito host not in the human blood smear. This is indicated line 181-182 'The effect of blood type on a series of mosquito life-history traits, namely (i) competence for *P. falciparum* (oocyst / sporozoite prevalence and intensity)', lines 196-198 'Oocyst prevalence (i.e. proportion of females harboring at least one oocyst on their midgut) and intensity (i.e. number of *P. falciparum* oocysts in the midgut of infected females) were determined at 8 dpbm' and lines 255-257 'Parasite prevalence (oocyst or sporozoite stages) and intensity (oocyst stage only) were analysed using Generalized Linear Mixed Models (GLMMs) with a binomial and a zero-truncated negative binomial error structure respectively'.*

*Therefore, as indicated lines 258-260 and 411-413 we included gametocytemia, blood type and the interaction of gametocytemia and blood type in our model. We would expect a positive correlation between parasite intensity in the mosquito and gametocytemia, although it is not always statistically significant when having only a few isolates as the relationship is not linear.*

23/ Line 352. Please add the "-" symbol to state the +/- se of the blood meal size  
*We only put "+se" as the bars in the figure 3D are only represented with the "+se" and not with the "-se".*

24/ Line 397. Please change "...influenced..." by "...influence...", use the present tense given that you already used the "did" auxiliary before the word  
*This was corrected (line 481).*

25/ Lines 503-504. Can you please rewrite the second part of this sentence. I am not sure I understand what you are trying to say. That is, what do you mean by not separating exposed-infected vs exposed-uninfected females you would not be able to measure the cost  
*Upon ingestion of an infectious blood meal there are two possible outcomes: (i) exposed females become infected by the parasites, with oocyst(s) observed in their midguts or (ii) females are not infected by the parasites and no oocyst are observed. In case we would have carried out individual follow-ups of females, we could have determined which category they belong to, however, we pooled females in cups (design A) or cages (design B) and therefore could not differentiate their status (exposed-infected or exposed-uninfected). To measure the cost of infection only we would need to have individual follow up and*

*analyze exposed-infected females only. By pooling the two categories we are measuring the cost of exposure to the parasite (concerning both exposed-infected and exposed-uninfected females) and not the cost of actual infection (concerning exposed-infected females). We have rewritten the sentence to clarify the meaning (lines 594-597).*

26/ Line 554. Please eliminate the comma "," after the word "survival"  
*The comma has been eliminated (line 647).*

27/ Lines 557-559. A better structure for this sentence would be:  
"Although blood type did not influence progeny development time, progeny from female fed on chicken blood was smaller than the progeny from females fed on all other vertebrates blood (Fig 6), which highlights the fitness cost of feeding on chicken blood.  
*The sentence was restructured as suggested (lines 650-652).*

28/ Line 561. After the parenthesis add a comma and continue with the work "but" instead of "although". It will read:  
"(..., Barreaux et al. 2016), but the local environmental..."  
*We corrected as requested (line 654).*

29/ Lines 566-595. I very much appreciate this last paragraph clearly showing the limitation and alternative explanations of your study. Yet, I would ask you to include a final paragraph where you bring all your study together in order to provide the take home messages of your study by discussing how your study results can help you better understand the local ecology of this host-vector-parasite system and what practical suggestions can you provide for the medical practioners.

For instance, with all the interesting results you have, one can start thinking on ways to implement malaria reducing strategies by seasonally changing the type of domestic animal that is used in local villages in the area. Say, during the peak of malaria transmission in the rainy season, villagers may be encourage to utilize more chickens than goats, or to keep goats in better protected shelters using treated nets or just nets to reduce the contact with mosquitoes. Based on your results, just that practical advice can work to reduce malaria seasonal incidence.

In other words, I would like to see a final paragraph where you link your results to the ecological reality of your study area.

*We have now added a final paragraph linking the results to the ecological reality of the study area (lines 691-714).*

## **Reviews**

***Reviewed by Francisco C. Ferreira, 01 Mar 2023 21:41***

*All changes made in response to the first reviewer's comments are highlighted in yellow in the revised version of the ms.*

The study by Vantaux and colleagues evaluated a broad spectrum of mosquito fitness traits that may be impacted by human infections with Plasmodium falciparum and host choice in subsequent blood meals. Through extensive experimental challenges using four different

blood sources in sequential feedings, the authors found that Plasmodium-infected mosquitoes had lower feeding rates compared to uninfected mosquitoes, an effect observed for all four blood types in the first feeding after Plasmodium exposure. Infection status did not affect any other trait, which were largely influenced by the host species of the blood source. Additionally, the blood source did not affect Plasmodium development within the mosquito. These results provided essential parameters to model vectorial capacity in multi-host environments. The introduction is comprehensive and provides a clear rationale behind the study. The manuscript is fairly well-written. However, below I have addressed some parts that could be further clarified. The laboratory methods used to evaluate the fitness traits are reliable, and the statistical analyses performed are robust.

30/ The authors used field isolates of *P. falciparum* and lab populations of *An. coluzzii* that were frequently replenished with wild-caught F1 mosquitoes, making the experimental set-up similar, but not identical to "natural" conditions. This is a major contribution of this study, but it would be important that the authors provide evidence that the replenishment of lab colonies with wild mosquitoes is sufficient to remove inbreeding effects that may affect mosquito-Plasmodium interactions. I am saying this because a substantial portion of the first paragraph of the 'Discussion' is dedicated to comparisons between natural and non-natural experiments. Although this was addressed in the "study limitations" section, the authors could tone down the assumption that they used a natural system.

*Although likely, we did not make sure that our mosquito colony was indeed outbred following frequent replenishment as we did not measure its genetic diversity. However, the studies compared to our work in the first paragraph of the discussion not only use long-term maintained mosquito colonies but also long-term in vitro cultured P. falciparum clones, which means that replicates were carried out on a single parasite clone. Overall, we agree with the reviewer that we cannot ascertain mosquito colony diversity and we have toned down the first paragraph of our discussion accordingly (lines 576-580).*

31/ It would be important to include the effect of blood meal size on the models evaluating mosquito survival, fecundity, development time and F1 wing length since the amount of blood ingested may play a role in these traits.

*We agree that the amount of blood ingested may play a role in mosquito survival, fecundity or F1 characteristics. However, blood meal size is an averaged size per cup which does not seem well suited to correlate to individual measurements of survival and F1 development time and wing length. We have added meal size as a covariate in the fecundity models and found no significant effect of the average blood meal size except on the average number of eggs and larvae from the first gonotrophic cycle and the hatching rate for blood meal 2 to 4 for which significant negative correlations were observed. Those results are now added lines 279, 481-510 and in the supplementary files.*

32/ It is unclear why the authors kept mosquitoes in cages for trials 3-5. Was it for convenience since the traits measured in these trials did not require close observation of individual mosquitoes? In any case, this could be clarified early in the 'Methods' section.



*Indeed, the replicates in cages did not require close observation of individual mosquitoes as we measured only mosquito competence. In the replicates in cups, the number of individuals available to measure competence at the oocyst and the sporozoite stages (119 and 34 individuals respectively) was rather reduced owing to both mortality and loss of individuals at each blood meal (unfed females were discarded) and the logistical constraints of feeding and following-up several cups in parallel. The replicates in cages allowed to follow much more females, and allowed us to screen 757 individuals at the oocyst stage and 235 individuals at the sporozoite stages, giving more robustness to our results. Following comment #5 from the recommender and this comment we have now made this clearer in the methods section (lines 141-147, 156-162 and 257-258).).*

33/ It's very interesting that feeding rate was the only trait affected by Plasmodium infection. However, does the experimental set-up (artificial feeding) allow authors to extrapolate this effect to real-world scenarios? I wonder if it would be better to use feeding-rate data from studies using whole-body to address feeding success. Even studies using immobilized hosts may provide more realistic information compared to the present study that used parafilm membrane for feeding. Using these parameters for the modeling is probably fine, though, since the cross-comparisons among blood types provide the relative impact of each host species.

*We agree with the reviewer and a sentence about this limitation has been added to the discussion (lines 671-674).*

34/ The authors explain only in the 'Discussion' why they did not sugar-feed mosquitoes in their experiments. It may be worth explaining this in the 'Methods' as well. In the 'Discussion', it would be interesting to see some explanation about how sugar-feeding may alleviate potential fitness costs in infected mosquitoes. Has this been demonstrated before? Ferguson et al 2003 (<https://doi.org/10.1111/j.0014-3820.2003.tb01521.x>) provide evidence that sugar restriction reduces survival in infected mosquitoes.

*We are now explaining this in the 'Methods' (lines 170-175) and in the 'Discussion' (lines 592-594).*

35/ L314 - Maybe the authors could clarify which prevalence is represented in the pie charts in Fig. 1, whether it's the oocyst or sporozoite rates.

*We have now specified that the pie charts represent oocyst prevalences (line 402).*

36/ L503-504 - What did authors mean by "that by not separating exposed-infected and exposed-uninfected females we were not able to measure them". Mosquito groups exposed to either infected or uninfected blood meals were mixed in the same cup/cage? Please clarify this in the 'Methods' and/or in the 'Discussion'.

*The first blood meal on infected human blood resulted in three categories:*

- *a non-infectious blood meal following heat-inactivation and giving an uninfected group*

- *an infectious blood meal resulting in exposed females becoming infected by the parasites with oocyst(s) observed in their midguts giving an exposed-infected group*
- *an infectious blood meal resulting in exposed females not becoming infected by the parasites for which no oocysts are observed. Giving an exposed-uninfected group*

*We had a set of cups or cages for the unexposed females (females which received a non-infectious blood meal) and a separate set of cups or cages for the exposed females (pooling exposed-infected and exposed-uninfected females). To measure the cost of infection only we would need to have individual follow up and analyze exposed-infected females only. By pooling the two categories we are measuring the cost of exposure to the parasite (concerning both exposed-infected and exposed-uninfected females) and the cost of infection (concerning exposed-infected females only). Following comment #25 from the recommender and this comment we have now clarified in the methods (lines 187-188) and the sentences in the discussion were rewritten (lines 594-597).*

***Reviewed by anonymous reviewer, 30 Mar 2023 01:25***

The article entitled "Multiple hosts, multiple impacts: the role of vertebrate host diversity in shaping mosquito life history and pathogen transmission" provides valuable information on the effect of blood-meals on the physiology, reproduction, survival and epidemiology of *Anopheles coluzzi*, particularly in the gonotrophic cycle. The authors acknowledge the limitations of a laboratory study, but provide bases for studies in natural environments. Therefore, **I recommend being accepted for publication in this journal.**

It is a complete and interesting article, I only have one suggestion:

37/ Figure 3 and Figure 6.- The colors cause me confusion, I recommend that in graphs B and D use gray scale or include the four solid or degraded colors in exposed and unexposed.

*We prefer to keep the color codes as they are used in all figures. We have used a stripped pattern instead of degraded colors to facilitate the visual assignation of the different groups.*

38/ Figure 2.- It provides relevant information, but it is small. I recommend separating or enlarging, to facilitate reading.

*We have now enlarged figure 2.*

Table results AIC values model selection related to question 15.

<i>Models are ordered by AIC values,</i>	<b>AIC values</b>
<i>When multiple models only the first 10 models with the lowest AIC are presented</i>	
<i>Models in bold were the minimal models by LRT</i>	
ANALYSIS COMPETENCE DATA REPLICATE 1 & 2	
<b>prev2=glmmTMB(infoo~carrier+(1 cup),data=comp1ooc, family=binomial)</b>	<b>159.1</b>
prev1=glmmTMB(infoo~blood+carrier+(1 cup),data=comp1ooc, family=binomial)	160.1
prev3=glmmTMB(infoo~1+(1 cup),data=comp1ooc, family=binomial)	164
prev4=glmmTMB(infoo~blood+(1 cup),data=comp1ooc, family=binomial)	166.6
<b>int1 &lt;- glmmTMB(oocysts~blood+carrier + (1 cup), data=comp1pos, family=truncated_nbinom2(link = "log"))</b>	<b>471.9</b>
int2 <- glmmTMB(oocysts~carrier + (1 cup), data=comp1pos, family=truncated_nbinom2(link = "log"))	476.4
int3 <- glmmTMB(oocysts~blood + (1 cup), data=comp1pos, family=truncated_nbinom2(link = "log"))	494.8
int4 <- glmmTMB(oocysts~1 + (1 cup), data=comp1pos, family=truncated_nbinom2(link = "log"))	494.4
<b>ps3=glmmTMB(infspz~1+(1 cup),data=comp1spz, family=binomial)</b>	<b>43.1</b>
ps2=glmmTMB(infspz~carrier+(1 cup),data=comp1spz, family=binomial)	43.1
ps1=glmmTMB(infspz~blood+carrier+(1 cup),data=comp1spz, family=binomial)	46.4
ps4=glmmTMB(infspz~blood+(1 cup),data=comp1spz, family=binomial)	47
ANALYSIS COMPETENCE DATA REPLICATES 3,4,5	
<b>prev4=glmmTMB(infoo~1+(1 replicate/carrier),data=comp2ooc, family=binomial)</b>	<b>829.4</b>
prev3=glmmTMB(infoo~gameto+(1 replicate/carrier),data=comp2ooc, family=binomial)	830
prev5=glmmTMB(infoo~blood+(1 replicate/carrier),data=comp2ooc, family=binomial)	832.5
prev2=glmmTMB(infoo~blood+gameto+(1 replicate/carrier),data=comp2ooc, family=binomial)	832.9
prev1=glmmTMB(infoo~blood*gameto+(1 replicate/carrier),data=comp2ooc, family=binomial)	837.6
<b>int1 &lt;- glmmTMB(oocysts~blood*gameto + (1 replicate/carrier), data=comp2pos, family=truncated_nbinom2(link = "log"))</b>	<b>4091</b>
int2 <- glmmTMB(oocysts~blood+gameto + (1 replicate/carrier), data=comp2pos, family=truncated_nbinom2(link = "log"))	4095.9
int4 <- glmmTMB(oocysts~gameto + (1 replicate/carrier), data=comp2pos, family=truncated_nbinom2(link = "log"))	4095.2
int5 <- glmmTMB(oocysts~1 + (1 replicate/carrier), data=comp2pos, family=truncated_nbinom2(link = "log"))	4098.2
int3 <- glmmTMB(oocysts~blood + (1 replicate/carrier), data=comp2pos, family=truncated_nbinom2(link = "log"))	4099
<b>prev4=glmer(infspz~1+(1 replicate/carrier),family=binomial,data=comp2spz)</b>	<b>249</b>
prev3=glmer(infspz~gameto+(1 replicate/carrier),family=binomial,data=comp2spz)	249.7
prev5=glmer(infspz~blood+(1 replicate/carrier),family=binomial,data=comp2spz)	252.6
prev2=glmer(infspz~blood+gameto+(1 replicate/carrier),family=binomial,data=comp2spz)	253.1
prev1=glmer(infspz~blood*gameto+(1 replicate/carrier),family=binomial,data=comp2spz)	256.7
ANALYSIS FEEDING SUCCESS REPLICATE 1&2, BLOOD MEAL 2 3 4	
m21=glmmTMB(cbind(nrfed,nrunfed)~feed+exposure+blood+ blood:feed+(1 cup),family=binomial,data=fs)	882.8
<b>m4=glmmTMB(cbind(nrfed,nrunfed)~blood+exposure+feed+replicate+blood:feed+(1 cup),family=binomial,data=fs)</b>	<b>883.6</b>
m22=glmmTMB(cbind(nrfed,nrunfed)~feed+exposure+blood + blood:feed+ exp:fed+(1 cup),family=binomial,data=fs)	886.7
m3=glmmTMB(cbind(nrfed,nrunfed)~blood+exposure+feed+replicate+blood:feed+ feed:exposure+(1 cup),family=binomial,data=fs)	887.6

m23=glmmTMB(cbind(nrfed,nrunfed)~feed+exposure+blood+ blood:feed+ exp:blood+(1   cup),family=binomial,data=fs)	888.4
m24=glmmTMB(cbind(nrfed,nrunfed)~feed+exposure+blood+ + replicate+ blood:feed+ exp:blood+(1   cup),family=binomial,data=fs)	889.2
m25=glmmTMB(cbind(nrfed,nrunfed)~feed+blood+ blood:feed+ (1   cup),family=binomial,data=fs)	889.6
m26=glmmTMB(cbind(nrfed,nrunfed)~feed+blood+ +replicate+ blood:feed+ (1   cup),family=binomial,data=fs)	890.4
m27=glmmTMB(cbind(nrfed,nrunfed)~feed+ exposure+blood+ blood:exposure+ exposure:feed+ blood:feed+blood:feed:exposure+ (1   cup),family=binomial,data=fs)	892
m28=glmmTMB(cbind(nrfed,nrunfed)~feed+ exposure+blood+ blood:exposure+ exposure:feed+ blood:feed+ (1   cup),family=binomial,data=fs)	892.3
ANALYSIS BLOOD MEAL SIZES	
analysis blood meal 2 3 4	
<b>mb4=glmmTMB(sizefinal~blood+exposure+feed+replicate+blood:feed+(1   cup), data=fs)</b>	<b>455</b>
mb3=glmmTMB(sizefinal~blood+exposure+feed+replicate+blood:feed+blood:exposure+(1   cup), data=fs)	456.3
mb2=glmmTMB(sizefinal~blood+exposure+feed+replicate+blood:feed+blood:exposure+exposure:feed+(1   cup), data=fs)	459.5
mb5=glmmTMB(sizefinal~blood+exposure+feed+replicate+(1   cup), data=fs)	465.5
mb1=glmmTMB(sizefinal~blood*exposure*feed+replicate+(1   cup), data=fs)	469
mb6=glmmTMB(sizefinal~blood+exposure+replicate+(1   cup), data=fs)	503.8
ANALYSIS SURVIVAL	
<b>s=coxme(Surv(death,status)~blood+replicate+(1   cup),data=s)</b>	<b>4343.2</b>
s=coxme(Surv(death,status)~blood+exposure+replicate+(1   cup),data=s)	4345.1
s=coxme(Surv(death,status)~blood+exposure+replicate+exposure:replicate+(1   cup),data=s)	4345.9
s=coxme(Surv(death,status)~blood+exposure+replicate+blood:exposure+(1   cup),data=s)	4348.5
s=coxme(Surv(death,status)~blood+replicate+blood:replicate+(1   cup),data=s)	4348.7
s=coxme(Surv(death,status)~blood+exposure+replicate+blood:exposure+exposure:replicate+(1   cup),data=s)	4349.5
s=coxme(Surv(death,status)~blood+exposure+replicate+blood:replicate+(1   cup),data=s)	4350.7
s=coxme(Surv(death,status)~blood+exposure+replicate+blood:replicate+exposure:replicate+(1   cup),data=s)	4351.6
s=coxme(Surv(death,status)~blood+exposure+replicate+blood:exposure+blood:replicate+(1   cup),data=s)	4354.1
s=coxme(Surv(death,status)~blood+exposure+replicate+blood:exposure+blood:replicate+exposure:blood+(1   cup),data=s)	4355.2
ANALYSES FECUNDITY	
egg prevalence egg lay 1	
m=glm(eggprev~replicate+exposure,family=binomial,data=fsr1)	47.7
m=glm(eggprev~exposure,family=binomial,data=fsr1)	48.6
m=glm(eggprev~replicate,family=binomial,data=fsr1)	48.8
m=glm(eggprev~replicate+exposure+exposure:replicate,family=binomial,data=fsr1)	49.7
<b>m=glm(eggprev~1,family=binomial,data=fsr1)</b>	<b>49.7</b>
analysis average nr of eggs, egg lay 2 3 4	
<b>mme21=glmmTMB(m2~replicate+(1   cup),data=dfs)</b>	<b>492.9</b>
mme21=glmmTMB(m2~feed+replicate+(1   cup),data=dfs)	494
mme21=glmmTMB(m2~exposure+replicate+(1   cup),data=dfs)	494.9

mme21=glmmTMB(m2~exposure+feed+replicate+(1   cup),data=dfs)	496
mme21=glmmTMB(m2~blood+replicate+(1   cup),data=dfs)	497.4
mme21=glmmTMB(m2~feed+blood+replicate+(1   cup),data=dfs)	499.3
mme21=glmmTMB(m2~exposure+blood+replicate+(1   cup),data=dfs)	499.4
mme21=glmmTMB(m2~1+(1   cup),data=dfs)	499.9
mme21=glmmTMB(m2~exposure+feed+blood+replicate+(1   cup),data=dfs)	501.3
mme21=glmmTMB(m2~exposure+(1   cup),data=dfs)	501.9
analysis mean nr of larvae egg lay 2,3 & 4	
<b>m1=glmmTMB(l2~feed+replicate+(1   cup),data=dfs2)</b>	<b>479.9</b>
m1=glmmTMB(l2~exposure+feed+replicate+(1   cup),data=dfs2)	480.4
m1=glmmTMB(l2~replicate+(1   cup),data=dfs2)	484.7
m1=glmmTMB(l2~feed+blood+replicate+(1   cup),data=dfs2)	484.8
m1=glmmTMB(l2~exposure+feed+blood+replicate+(1   cup),data=dfs2)	485.2
m1=glmmTMB(l2~exposure+replicate+(1   cup),data=dfs2)	486.2
m1=glmmTMB(l2~+blood+replicate+(1   cup),data=dfs2)	488
m1=glmmTMB(l2~exposure+blood+(1   cup),data=dfs2)	489.3
m1=glmmTMB(l2~feed+(1   cup),data=dfs2)	500.1
m1=glmmTMB(l2~exposure+feed+(1   cup),data=dfs2)	501.2
ANALYSIS F1 DEVELOPMENT TIME EGG LAY 1	
<b>m1=coxme(Surv(dvlptime,status)~1+(1   cup),data=fp1)</b>	<b>4777.6</b>
m1=coxme(Surv(dvlptime,status)~sex+(1   cup),data=fp1)	4778.3
m1=coxme(Surv(dvlptime,status)~exposure+(1   cup),data=fp1)	4778.4
m1=coxme(Surv(dvlptime,status)~exposure+sex+(1   cup),data=fp1)	4779.1
m1=coxme(Surv(dvlptime,status)~exposure*sex+(1   cup),data=fp1)	4780.5
Analysis F1 developing time egg lay 2, 3, 4	
model=coxme(Surv(dvlptime,status)~blood+egglay+sex+(1   cup),data=fp234)	6043
model=coxme(Surv(dvlptime,status)~blood+egglay+sex+density+(1   cup),data=fp234)	6043.2
<b>model=coxme(Surv(dvlptime,status)~egglay+sex++(1   cup),data=fp234)</b>	<b>6043.3</b>
model=coxme(Surv(dvlptime,status)~egglay+sex+density+(1   cup),data=fp234)	6043.5
model=coxme(Surv(dvlptime,status)~blood+egglay+exposure+sex+(1   cup),data=fp234)	6043.8
model=coxme(Surv(dvlptime,status)~egglay+exposure+sex+(1   cup),data=fp234)	6043.9
model=coxme(Surv(dvlptime,status)~blood+egglay+exposure+sex+density+(1   cup),data=fp234)	6044
model=coxme(Surv(dvlptime,status)~egglay+exposure+sex+density+(1   cup),data=fp234)	6044.1
model=coxme(Surv(dvlptime,status)~blood+egglay+(1   cup),data=fp234)	6044.9
model=coxme(Surv(dvlptime,status)~blood+egglay+density+(1   cup),data=fp234)	6045.3
Analysis F1 size Egg lay 1	
<b>m1=glmmTMB(size~sex+(1   cup),data=s1)</b>	<b>-167.5</b>
m1=glmmTMB(size~exposure+sex+(1   cup),data=s1)	-166.7
m1=glmmTMB(size~exposure*sex+(1   cup),data=s1)	-164.9

m1=glmmTMB(size~1+(1 cup),data=s1)	-101.1
m1=glmmTMB(size~exposure+(1 cup),data=s1)	-100.7
Analysis F1 size Egg lay 2, 3, 4	
<b>m1=glmmTMB(size2~blood+sex+egglay+density+(1 cup),data=s2)</b>	<b>1316.5</b>
m1=glmmTMB(size2~blood+sex+egglay+exposure+density+(1 cup),data=s2)	1314.9
m1=glmmTMB(size2~sex+egglay+density+(1 cup),data=s2)	1309.6
m1=glmmTMB(size2~blood+sex+egglay+(1 cup),data=s2)	1308.9
m1=glmmTMB(size2~blood+sex+egglay+exposure+(1 cup),data=s2)	1307.9
m1=glmmTMB(size2~sex+egglay+exposure+density+(1 cup),data=s2)	1307.6
m1=glmmTMB(size2~sex+egglay+(1 cup),data=s2)	1301.2
m1=glmmTMB(size2~sex+egglay+exposure+(1 cup),data=s2)	1299.5
m1=glmmTMB(size2~blood+sex+density+(1 cup),data=s2)	1290.7
m1=glmmTMB(size2~blood+sex+exposure+density+(1 cup),data=s2)	1288.7